TECHNICAL REPORT

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GLYCERDL INTERFERENCE IN THE DETERMINATION OF CRUDE FIBER, FAT, AND MOISTURE IN INTERMEDIATE MOISTURE MEAT PRDDUCTS

by

Lloyd Cox

August 1972

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

Food Laboratory

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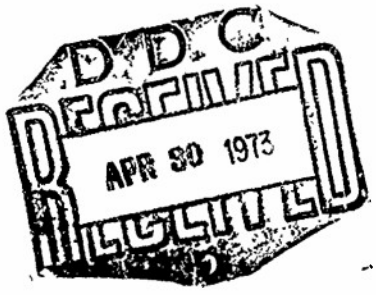

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13. ABSTRACT

The AOAC crude fiber method for animal feed was modified and evaluated for application to intermediate moisture meat products; where the AOAC method for crude fiber gave extremely high results, the modified method gave values in the expected range. The AOAC Mojonnier (mixed ether) method for fat in baked or expanded pet food was modified and compared to the Mojonnier (petroleum ether only) and the AOAC Soxhlet method (petroleum ether) for meat; the Mojonnier (mixed ether) method gave higher fat values than the other two methods but there was no evidence of extracted carbohydrates. The Karl Fischer moisture method was compared to two oven methods and the AOAC toluene distillation method for moisture in animal feed; the Karl Fischer results were comparable to the AOAC toluene distillation results. The four moisture methods were compared for results on different days; the first day results were comparable to the second day results.

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FOREWORD

One of the responsibilities of the Food Chemistry Division's Analytical Group is development of new chemical methods of analysis, or modification of existing ones for new and experimental food products.

When the high glycerol content of intermediate moisture meat products caused interference problems in the determinations of crude fiber, fat, and moisture, this study was conducted to evaluate the applicability of related AOAC* chemical methods. The results showed that selection and modification of existing methods of chemical analysis of food eliminated the interference problem.

This work was performed under Project 1J662713A034, Military Food Service and Subsistence Technology.

Grateful acknowledgement is accorded personnel of these Laboratories: PFC John R. Troy and Mr. Otto J. Stark for their assistance in the statistical analysis of the data obtained, and Mr. Albert S. Henick, Dr. Maxwell C. Brockmann, and Dr. J. Walter Giffee for their review of the manuscript.

*Official Methods of Analysis (1970) 11th Ed., Association of Official Analytical Chemists, Washington, D.C., 1970.

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ABSTRACT

The AOAC crude fiber method for animal feed was modified and evaluated for application to intermediate moisture meat products; where the AOAC method for crude fiber gave extremely high results, the modified method gave values in the expected range. The AOAC Mojonnier (mixed ether) method for fat in baked or expanded pet food was modified and compared to the Mojonnier (petroleum ether only) and the AOAC Soxhlet method (petroleum ether) for meat; the Mojonnier (mixed ether) method gave higher fat values than the other two methods but there was no evidence of extracted carbohydrates. The Karl Fischer moisture method was compared to two oven methods and the AOAC toluene distillation method for moisture in animal feed; the Karl Fischer results were comparable to the AOAC toluene distillation results. The four moisture methods were compared for results on different days; the first day results were comparable to the second day results.

INTRODUCTION

The continuous development of new food products for the combat soldier often requires modification of present food chemistry methods as well as the development of new methods to overcome interfering components and give reliable and meaningful test data.

The infusion of glycerol (at the level of about 30% of the product) into meat products to increase stability and shelf-life produces interference problems in the determinations of crude fiber, fat, and moisture. In the analysis of crude fiber, glycerol was removed from the sample before the crude fiber was determined. In the Mojonnier fat determination, glycerol and other ethyl ether-soluble components were removed by washing the mixed ether extracts with water. In the determination of moisture, the Karl Fischer method was compared to two oven methods and the toluene distillation method for its applicability to the intermediate moisture products.

This study was conducted to evaluate chemical methods for application to intermediate moisture meat products.

EXPERIMENTAL

The Association of Official Analytical Chemists (AOAC), **Official Methods of Analysis, 11th Edition, Chapters:** Meat and Meat Products and Animal Feeds (1) was followed, with modifications, in the development of these methods.

The intermediate moisture samples were furnished by the Animal Products Division, Food Laboratory, US Army Natick Laboratories.

The samples were blended in a Waring* blender (Model CB-6 with small jar adapter) and stored in jars with air-tight screw caps.

Three intermediate moisture samples of ham and raisin sauce were analyzed for crude fiber by the AOAC method and the results compared to those obtained with a modification of the method.

Two intermediate moisture samples of pork with sweet and sour sauce were analyzed for fat by the modified AOAC Mojonnier method (mixed ether extracts washed in a second Mojonnier extraction tube with water) and compared to the modified Mojonnier method (petroleum ether only) and the AOAC Soxhlet method (petroleum ether) for meat.

*Mention of company or trade names in this report does not imply endorsement over others not named.

A variety of intermediate moisture samples were analyzed for moisture by the Karl Fischer method (2, 3) and compared to the AOAC Animal Feed moisture method *modified* (vacuum oven, 16 hours at 70°C), the AOAC Meat and Meat Products moisture method (mechanical convection oven, 16-18 hours at 100-102°C), and the AOAC Animal Feed toluene distillation moisture method.

The four moisture methods were subjected to comparison of results on different days.

Method for Crude Fiber

When meat product samples containing large amounts of glycerol (about 30%) gave unusually high crude fiber values, a method was developed to remove glycerol from the sample before the crude fiber was determined. The modified method was compared to the AOAC method (7.053–7.057).

Apparatus and Reagents

(a) AOAC 7.054 and 7.055.
(b) Glassware – Centrifuge tubes, 250 ml; beakers, 600 ml; stirring rods, 7 inches long; mortar and pestle.
(c) Centrifuge – With head for 250 ml centrifuge tubes.
(d) Steam bath.
(e) Diethyl ether – Anhydrous, ACS grade.
(f) Ethyl alcohol – 95%, USP grade.

Procedure

Weigh 5-10g sample into 250 ml centrifuge tube. Extract (mix with stirring rod) with 75 ml ether, centrifuge, and decant to remove fat. Repeat the procedure. (Some glycerol is also removed.) Warm residue on steam bath with stirring to expel some of the ether. Add 50 ml of hot distilled water and heat on steam bath 10-15 minutes; add 50 ml alcohol, stir, centrifuge, and decant. Make two water-alcohol extractions of the residue to remove the glycerol. Transfer residue to 600 ml beaker using alcohol wash bottle. Evaporate alcohol on steam bath or decant alcohol from 600 ml beaker and dry residue in 100°C oven. (Residue should be dry and crunchy.) Break up residue with flat-end stirring rod or grind in mortar and pestle. Determine crude fiber as in AOAC 7.053-7.057.

Results and Discussion

The data in Table 1 compare the AOAC crude fiber method with the modified method. The AOAC method for crude fiber analysis gave results 18-29 times higher than what is considered normal for ham and raisin sauce whereas the modified method provided values in the expected range.

The high values obtained from the AOAC crude fiber method were probably due to aldehyde crosslinking (tanning) in the samples by acrolein (4,5) when samples containing glycerol were dried (100-125°C in mechanical convection oven 1-2 hours) prior to removal of fat.

Method for Fat (Modified Mojonnier)

The modifications made to the AOAC Mojonnier method were: (1) larger sample size digested in beaker (2) elimination of use of alcohol (3) washing mixed ether extracts with water in a second Mojonnier extraction tube (flask) and (4) pouring the water-washed mixed ether extracts directly into a tared 150 ml beaker. The Mojonnier method, where only petroleum ether was used did not require the water washing step.

Larger samples (10g) were used in the Soxhlet method for fat (AOAC 24.005), and petroleum ether was used instead of ethyl ether.

Procedure

Weigh 3-5g into 50 ml beaker and make paste with water (2-4 ml). Add 8-10 ml of conc. HCl (digest sample with 8N HCl if cereal content is high) and mix immediately with stirring rod. Cover beaker with watch glass and digest sample on steam bath for 30-45 minutes with occasional stirring. Transfer digested sample into Mojonnier extraction tube with distilled water and add sufficient water to reach the pouring-off level. Add 25 ml ethyl ether (in two 12.5 ml increments) into 50 ml beaker with ether wash bottle to extract traces of fat residue and transfer into Mojonnier extraction tube. Stopper Mojonnier tube with cork and mix gently. Carefully release pressure and wash cork with a few ml of petroleum ether using wash bottle. Add 25 ml of petroleum ether (in two 12.5 ml increments) into 50 ml beaker and transfer into Mojonnier tube. Stopper Mojonnier tube, shake, and centrifuge (600 rpm) for 3-5 minutes. Transfer mixed ether extract (use small funnel) into second Mojonnier tube containing water, stopper, shake, centrifuge, and transfer water-washed extract into tared 150 ml beaker. (Crystallizing dish, 80 x 40 mm. may be used instead of 150 ml beaker.) Evaporate extract in forced-draft hood or on steam bath. Make three additional extractions by pouring 25 ml of each ether directly into the first Mojonnier tube and following the same sequence of steps. (All transfer steps are made in a quantitative manner.) After the last extract evaporates, dry extracted fat in a mechanical convection oven at 100°C to constant weight (1.5-2 hrs.), cool in desiccator, and weigh.

Results and Discussion

The data in Table 2 permit comparison of fat recovered by the three methods. The mixed ether Mojonnier method with water-washing in a second Mojonnier extraction tube gave the highest recoveries of fat, but no charred material (evidence of extracted carbohydrate, etc.) was found in the extracted fat.

In a similar study by Kuhn (6), the fat recovered by the Mojonnier method was higher than fat recovered by the Soxhlet method (petroleum ether). The Kuhn study also showed that, when the residue from the Soxhlet method was subjected to the Mojonnier method and the percent fat recovered was added to the percent fat by the Soxhlet method, the two fat values (Mojonnier value compared to total value of Soxhlet plus additional Mojonnier of Soxhlet residue) agreed reasonably well.

The data in Table 2 were subjected to analysis of variance, and the F-values showed significant difference at 0.05 probability when the Mojonnier method using mixed ether was compared to the Mojonnier method using petroleum ether only or the Soxhlet method using petroleum ether.

Method for Moisture (Karl Fischer)

The Karl Fischer method for moisture (2,3) was applied to intermediate moisture meat samples.

Apparatus and Reagents

(a) Karl Fischer Aquametry Apparatus – Labindustries, Berkeley, CA 94710.
(b) Oster blender – With blending assembly for standard Mason jar.
(c) Mason jar – 1/2 pint.
(d) Karl Fisher Reagent (KFR) – Harleco 3786, Arthur H. Thomas Co., Philadelphia, PA 19105.
(e) Methanol – Anhydrous.
(f) Standard – Weight 1g H_2O, to nearest mg, into 100 ml volumetric flask and bring to volume with methanol. Use flask short enough to fit on analytical balance, and cap flask with aluminum foil while weighing to prevent loss of H_2O. Titrate 5 ml aliquot of standard to determine KFR equivalence (eq.).

Procedure

Weight 5g sample into 1/2 pint Mason jar; immediately add 95 ml of methanol, and cap jar. Attach Oster blending assembly to jar and blend sample three 10 second intervals. Allow sample to settle in tightly capped jar. Blank for sample should be determined on 95 ml of methanol blended with dry blending assembly in the same manner as sample. Equilibrate Karl Fischer (KF) apparatus by adding sufficient methanol and a slight excess of KFR with stirring for about 15 minutes. Add enough 98% methanol (a few drops) to give a slight excess of H_2O, and titrate to exact KF end-point. Pipette 5 ml of standard, sample, or blank into KF cell, and titrate to KF end-point. Empty cell by aspiration. $\%H_2O$ = (ml KFR – blank) x KFR eq. x 100/mg sample, where mg sample = 5g x 1000/100 ml x 5 ml = 250 mg.

Results and Discussion

The data in Tables 3-6 show that the Karl Fischer method compared favorably to the toluene distillation method, with the exception of the values in Table 6.

The values for the vacuum oven method were slightly higher than the values for the Karl Fischer method and the toluene distillation method. The higher values were due partly to evaporation of acetic acid from the sample. Entrainment (extremely fine drops carried away with the vapor) of glycerol from the samples was indicated by condensation on the vacuum oven door.

The values for the mechanical convection oven method were extremely high compared to the other three methods. The data suggest that entrainment of glycerol from the samples is greater at atmospheric pressure than at reduced pressure.

Newman (7) observed the problem of loss of glycerol on 24 hours of drying glycerol solutions at reduced pressure and recommended Karl Fischer moisture determination for complex mixtures containing glycerol. Lawrie (4) observed that when distilling dilute glycerol solutions, loss due to entrainment was much greater at atmospheric pressure than at reduced pressure.

The data in Tables 3-6 were subjected to analysis of variance. The F-values comparing the toluene distillation method to the Karl Fischer method indicated insignificant difference at probability 0.05 for each product except Hong Kong Pork (Table 6) where the mean value for the Karl Fischer method is about 0.8% higher than the mean value for the toluene distillation method. The F-values comparing the mechanical convection oven and the vacuum oven method to the Karl Fischer method indicated significant differences at probability 0.05 in each product tested.

In Table 7 the four moisture methods were compared for results on different days. The data were subjected to analysis of variance. The F values comparing the first day to the second day indicated insignificant differences in each method except the vacuum oven method where at 0.05 probability there was slight significant difference.

CONCLUSIONS

When fat and glycerol were removed from the intermediate moisture meat product as described in this report, low and meaningful test results were obtained for crude fiber.

The Mojonnier fat method (mixed ether) gave the highest test results, and the water washing eliminated interference of glycerol, carbohydrates, etc. The method can probably be applied to semi-moist pet food products and other food products containing ethyl ether soluble components (glycol, glycerol, carbohydrates, lactic acid, sorbate, etc.)

The Karl Fischer and toluene distillation methods were equally good for determination of moisture in intermediate moisture meat products. The vacuum oven and mechanical convection oven methods gave high results, especially the latter method. The vacuum oven method is not recommended, but it can be used if consideration is made for evaporation of acetic acid and entrainment of a small percent of glycerol from the sample.

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Table 1. Comparative Crude Fiber (CF) Results of Ham and Raisin Sauce

Sample #	% CF, AOAC	% CF, MODIFIED AOAC
03259	6.72	0.36
	6.72	0.39
03260	5.47	0.28
	5.74	0.24
03261	5.71	0.20
	5.30	0.18

Table 2. Comparative Fat Results (%) of Pork with Sweet and Sour Sauce

	Sample #12217			Sample #12218		
	Mojonnier, Mixed Ether	Mojonnier, Pet Ether	Soxhlet, Pet Ether	Mojonnier, Mixed Ether	Mojonnier, Pet Ether	Soxhlet, Pet Ether
	5.15	4.82	4.83	5.59	5.31	5.29
	5.11	4.75	4.80	5.57	5.20	5.26
	4.95	4.86	4.82	5.43	5.27	5.29
	4.98	4.83	4.87	5.39	5.25	5.29
	5.01	4.89	4.83	5.38	5.32	5.26
Mean	5.04	4.83	4.83	5.47	5.27	5.27
Std. Dev.	0.09	0.05	0.02	0.10	0.05	0.01
F value		21.39	27.39		16.36	18.11

Table 3. Comparative Moisture Results (%) of Pork with Sweet and Sour Sauce

	Karl Fischer	Vacuum Oven	Mechanical Convection Oven	Toluene Distillation
	38.02	38.54	45.74	38.0
	37.90	38.78	45.12	38.0
	37.54	38.89	46.32	38.2
	37.78	39.53	46.39	37.8
	38.02	39.42	45.26	
	38.25	39.37	46.71	
Mean	37.91	39.10	45.92	38.0
Std. Dev.	0.24	0.40	0.65	0.16
F-value		36.93	799.0	0.34

Table 4. Comparative Moisture Results (%) of Barbecue Beef

	Karl Fischer	Vacuum Oven	Mechanical Convection Oven	Toluene Distillation
	42.03	43.45	48.60	41.0
	41.91	43.06	49.33	41.5
	42.26	43.61	47.36	42.0
		43.66	48.28	41.5
		43.85	48.81	
		43.85	47.89	
Mean	42.07	43.58	48.38	41.5
Std. Dev.	0.18	0.30	0.70	0.41
F-value		63.71	224.1	4.89

Table 5. Comparative Moisture Results (%) of Ham and Raisin Sauce

	Karl Fischer	Vacuum Oven	Mechanical Convection Oven	Toluene Distillation
	41.68	43.79	48.93	42.0
	41.79	43.37	48.96	41.8
	42.15	44.21	50.92	42.7
	41.91	44.08	51.86	42.5
	42.15	43.34	50.60	
		43.46	50.85	
		42.93	51.86	
		42.88	50.94	
Mean	41.94	43.51	50.62	42.3
Std. Dev.	0.21	0.49	1.13	0.42
F-value		45.02	279.81	2.16

Table 6. Comparative Moisture Results (%) of H ng Kong Pork

	Karl Fischer	Vacuum Oven	Mechanical Convection Oven	Toluene Distillation
	42.62	42.90	48.12	42.0
	42.50	42.89	47.70	41.5
	42.62	43.00	50.60	42.0
	42.62	43.14	51.02	41.5
	42.59	43.04	50.70	41.5
	43.17	43.15	50.01	42.0
	42.82	43.27	50.58	41.5
		43.54	49.55	42.0
				41.5
				42.0
Mean	42.59	43.12	49.79	41.8
Std. Dev.	0.06	0.21	1.25	0.29
F-value		22.18	126.72	32.52

Table 7. Comparison of Moisture Results (%) on Different Days

	Pork with Sweet and Sour Sauce						Hong Kong Pork	
	Karl Fischer		Vacuum Oven		Mechanical Convection Oven		Toluene Distillation	
	1st day	2nd day	1st day	2nd day	1st day	2nd day	1st day	2nd day
	37.66	37.90	38.54	39.81	45.74	44.06	42.0	41.5
	38.02	37.78	38.78	39.33	45.12	44.46	41.5	42.0
	37.54	38.25	38.89	39.11	46.32	44.17	42.0	41.5
	37.42		38.53	38.99	46.39	46.08	41.5	42.0
	38.02		39.37	40.02	45.26	45.88		41.5
			39.42	39.69	46.71	45.31		42.0
Mean	37.73	37.98	38.92	39.49	45.92	44.99	41.8	41.8
Std. Dev.	0.28	0.24	0.39	0.41	0.65	0.88	0.29	0.27
F-values	1.59		6.05		4.32		0.00	